
Recent clinical data have shown that early plasmin proteolysis of human fibrinogen is often the release of fibrinopeptide B (FPB) immunoreactivity into plasma. Upon thrombin treatment this activity increases indicating that the immunoreactivity arises from larger FPB-containing peptides. The presence of degradation products of the B8 chain may be an index of fibrinogen proteolysis not only in normal hemostasis but also in pathological disorders such as thrombosis and disseminated intravascular coagulation. Therefore the capability of detecting events leading to progressive plasmin degradation at the B8 chain could be of major diagnostic and therapeutic significance. Toward this goal, we have isolated the various peptides released from human fibrinogen by plasmin proteolysis using HPLC on a reverse phase C18 column. These peptides were identified using amino acid analysis and radioimmunoassays for FPB. B8 1-42 was the earliest fragment released during limited plasmin proteolysis. These levels reached a maximum and then began to fall during the course of the digestion. In addition, increasing levels of B8 1-21 and of FPB were shown to lag behind the production of B8 1-42. Using purified B8 1-42 as a potential substrate was shown to occur at the 21-22 bond, with a minor cleavage at the 14-15 bond. Exhaustive digestion yielded two major components, B8 1-42 and B8 22-42 and a minor component composed of B8 15-42. The rate of this reaction is not affected by the addition of hirudin indicating that it is not caused by trace amounts of thrombin. Taken together these data indicate a sequence of events in which B8 1-42 is initially cleaved from fibrinogen during plasm digestion. Free from its parent molecule, B8 1-42 can undergo further plasmin attack, preferentially at the 21-22 bond to yield B8 1-21 and B8 22-42. Plasmin then attacks B8 1-21 at the 14-15 bond to release FPB.

A NEW, RAPID AND SENSITIVE DETERMINATION OF FIBRINOPEPTIDE A (FPA) BY A HIGH AFFINITY ANTIBODY TO FPA ANTISERUM. G. Stahle, J. Harenberg, H. Schmidt-Gayk and D. Zimmermann. Department of Clinical Pharmacology and Internal Medicine, University of Heidelberg, GFR

The clinical relevance of the determination of FPA is not yet fully recognized because of the small time consumption which is still required. Shortening of the incubation times always lead to a critical loss of the sensitivity of the assay. We present now a modification which provides highly sensitive and reproducible results within 2 hrs.

The ethanolic extraction of FPA from plasma was shortened to 20 min including centrifugation. Samples were analysed in triplicates and evaporated at 50°C on microtiter plates. The addition of 0.2ul normal rabbit serum (NRS) lead to a 20% increase of the reaction rate of the FPA antiserum to FPA. A second antibody with high affinity to rabbit immunoglobulin i.e. the FPA antiserum improved the maximal binding to 35% after an incubation period of only 10 min. 35-40000 cpm tracer FPA were added to each sample. FPA antiserum, second antibody and NRS were preincubated for 1-24 hrs and then added together with the tracer to the samples. Thus a sensitive standard curve was obtained between 0.16 and 160 ng/ml (12000-600 cpm). The correlation coefficient of this modification to our previously described method was r=0.98 (n=60) The variation coefficient could be improved substantially to 3.1 for low, 4.0 for medium and 4.4 for high FPA. Normal FPA was between 0.16 and 2.5 ng/ml (mean 1.4 ng/ml, n=32). The presented modification of the radioimmunoassay determines the disadvantages of previously described methods and provides acute results within 2 hrs.